American Journal of Medical Genetics 70:43–47 (1997)

Down Syndrome With Biparental Inheritance of der(14q21q) and Maternally Derived Trisomy 21: Confirmation by Fluorescent In Situ Hybridization and Microsatellite Polymorphism Analysis

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Individuals with translocation Down syndrome (DS) often inherit the rearranged chromosome from a carrier parent. DS due to inheritance of one Robertsonian or derivative (14q21q) from one parent and a second der(14q21q) in addition to a free chromosome 21 from the other parent are rarely documented in liveborn infants. Presented here is such a propositus with DS and with a unique karyotype 45,XY,der(14;21)(p11.1;p11.1)pat,der(14;21)(p11.1;q11.1)mat, +21mat. Using conventional chromosome heteromorphisms, fluorescent in situ hybridization (FISH), and microsatellite polymorphism analyses, we established the biparental origin of the 2 der(14q21q) and the maternal origin of the extra chromosome 21 in the patient. A combination of both cytogenetic and molecular genetic techniques also enabled us to show that the 2 der(14q21q) were not identical by descent and hence the parents were nonconsanguineous. It has been a well-established fact that mothers with Robertsonian translocations have higher risk for nondisjunction than do carrier fathers. Our case, wherein the nondisjunctional event occurred in the mother, even though both parents are carriers of a 14;21 Robertsonian translocation, is yet another example of this. Am. J. Med. Genet. 70:43–47, 1997. © 1997 Wiley-Liss, Inc.

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Received 13 February 1996; Accepted 22 July 1996

KEY WORDS: Down syndrome; Robertsonian translocation; nondisjunction; in situ hybridization; microsatellite polymorphism; parental origin

INTRODUCTION

A remarkable degree of karyotype variation is known to occur in Down syndrome (DS). DS individuals with 2 Robertsonian translocations (14q21q) and a single free chromosome 21 have occasionally been reported [Borgaonkar, 1993]. Cytogenetic heteromorphisms and restriction fragment length polymorphism (RFLP) studies have shown that in about 80–90% of patients with free trisomy, the extra chromosome arises due to nondisjunction in the mother [Stewart et al., 1988; Brahe et al., 1990; Bricarelli et al., 1990; Takaesu et al., 1990; Antonarakis et al., 1992]. More recently, microsatellite polymorphism analysis has been shown to be highly effective in determining the parental origin of the extra chromosome 21 in translocation DS as well as in simple trisomy 21 [Shaffer et al., 1993; Zhao et al., 1994]. Documented here is a DS individual with 2 Robertsonian translocations, or 2 der(14q21q) chromosomes along with an accessory chromosome 21. Conventional cytogenetic heteromorphism and fluorescent in situ hybridization (FISH) analyses indicated that the 2 der(14q21q) chromosomes were biparental in origin. Using polymerase chain reaction (PCR) analysis with a panel of polymorphic microsatellite markers from chromosomes 14 and 21, we have further established the biparental origin of the 2 der(14q21q) chromosomes and the maternal origin of the extra chromosome 21. Further, using a combination of molecular cytogenetic and molecular genetic methodologies, we documented that the 2 der(14q21q) chromosomes were not identical

TABLE I. Microsatellite Markers Used in PCR Analysis

Marker	Chromosome band	Marker	Chromosome band
D21S13E	21q11	PFKL	21q22.3
D21S120	21q11	D21S268	21q22.2-qter
APP	21q21.2	D21S270	21q22.2-qter
IFNAR	21q22.1	D21S1259	21q22.3
D21S156	21q22.3	D21S171	21q22.3
TCRD	14q11.2		

by descent and, thus, the nonconsanguineous nature of the relationship.

CLINICAL REPORT

The propositus, a male Asian Indian infant, was the product of the third pregnancy of nonconsanguineous parents. The mother's first pregnancy resulted in a spontaneous abortion at 4 months of gestation. The second pregnancy resulted in the birth of a phenotypically normal female infant. The mother and father were 24 and 33 years old, respectively, at the time of conception of the propositus. Pregnancy was uncomplicated. At term, the infant was delivered by cesarean section. Antenatal history was uneventful. On genetic evaluation at age 6 months, his length was 59 cm and weight was 5 kg (both >5th centile), head circumference was 42 cm (10th centile). The infant's phenotype was consistent with classical DS.

MATERIALS AND METHODS

Chromosome Analysis

Chromosome analyses of cultured blood lymphocytes from the propositus and parents were carried out as per the standard methods.

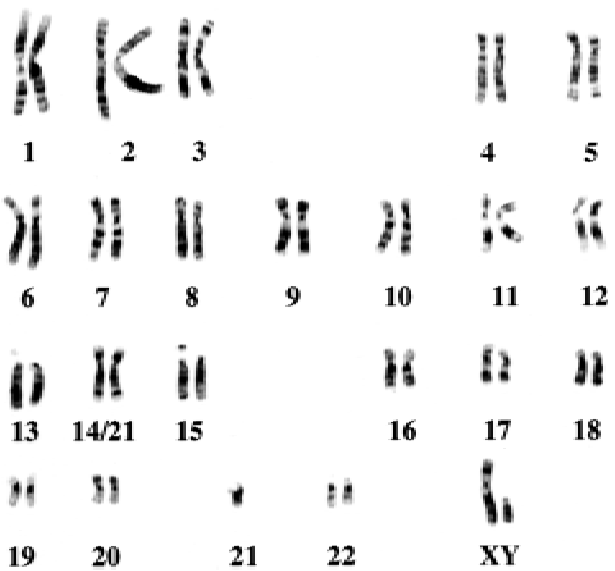

Fig. 1. Karyotype of the propositus.

In Situ Hybridization

In situ hybridization was performed using alpha satellite probes for the pericentromeric regions of chromosomes 13/21(D13Z1/D21Z1) and 14/22(D14Z1/D22Z1) from Oncor, Inc. Probe preparation, hybridization, posthybridization washing, and detection were carried out as per the instructions of the manufacturer. Alpha satellite probes for chromosomes 13/21 were labeled with digoxigenin and detected with rhodamine, while probes for chromosomes 14/22 were labeled with biotin and detected with fluorescein. DAPI counterstained chromosomes were analyzed under a Zeiss Axiophot microscope equipped with epifluorescence and triple band pass filters. Representative photographs were taken using Kodak Ektachrome 400 ASA film.

Microsatellite Polymorphism Analysis

Genomic DNA extracted from the peripheral blood of the propositus, his parents, and his sib were used for polymorphism analysis. Primers for the microsatellite markers were obtained either from Isogen Bioscience (Netherlands) or from Dr. Leslie Biesecker, NIH (Table I). The markers were amplified by PCR using a Perkin/Elmer 9600 thermocycler. Each reaction contained 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$,

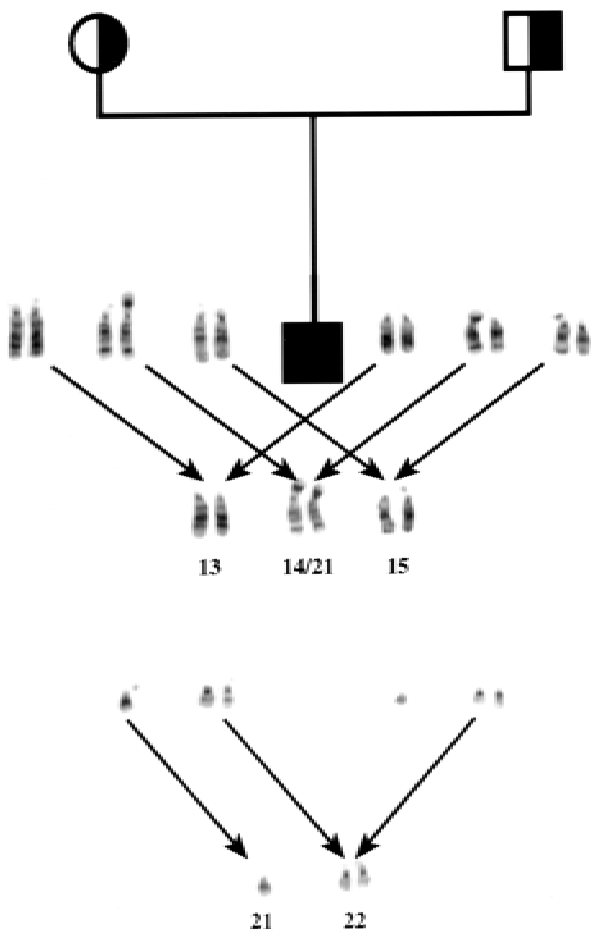

Fig. 2. Demonstration of the maternal origin of extra chromosome 21 using conventional cytogenetic heteromorphisms.

40 ng of each primer, 200 mM dATP, dGTP, and dTTP, 2.5 mM unlabeled dCTP, 16.7 nM P^{32}-labeled dCTP (3,000 Ci/mmol), and 0.45 units of Amplitaq DNA polymerase (Perkin-Elmer/Cetus), in a total volume of 15 μl. Samples were initially denatured at 94°C for 5 minutes, followed by 27 cycles of denaturing at 94°C for 1 minute, annealing at 55°C for 2 minutes, and extension at 72°C for 1 minute. The final extension was for 6 minutes at 72°C. The amplified products were resolved on a 6% acrylamide/7 M urea gel and visualized by autoradiography with XAR (Kodak) film.

RESULTS

Chromosome analysis from peripheral blood lymphocytes of the propositus showed each cell with 45 chromosomes, including 2 Robertsonian translocations [der(14q21q)] and an accessory chromosome 21 contributing to trisomy 21 with a karyotype 45,XY,der (14q21q),der(14q21q),+21 (Fig. 1). Parental chromosome analyses showed that the mother had a 45,XX,der(14q21q) and the father had a 45,XY,der (14q21q) chromosome complement. A phenotypically normal sister and the maternal grandmother of the propositus were also balanced translocation carriers with 45,XX,der(14q21q) karyotype. The maternal grandfather had a normal 46,XY karyotype. The paternal grandparents were not available for study.

With regard to the conventional cytogenetic heteromorphisms, the propositus and his relatives were very informative on all acrocentric chromosomes. A comparison of chromosome 21 heteromorphism clearly showed that the extra chromosome 21 in the propositus was maternal in origin (Fig. 2).

In situ hybridization (FISH) with alpha satellite DNA probes for chromosomes 14 (D14Z1/D22Z1) and 21 (D13Z1/D21Z1) on the propositus and his parents yielded the following results. In the father, FISH with D14Z1 showed signals on the normal chromosome 14 and on the der(14q21q). With the D21Z1 probe, the free chromosome 21 showed normal hybridization along with a signal on the der(14q21q) chromosome (Fig. 3A). Thus, the derivative chromosome in the father was dicentric.

In the mother, FISH with D14Z1 yielded signals on the normal 14 and der(14q21q). With probe D21Z1, the normal chromosome 21 showed hybridization while a signal was absent on the der(14q21q) (Fig. 3B). The der(14q21q) chromosome in the mother, therefore, was monocentric.

FISH with D14Z1 on the propositus showed both der(14q21q) chromosomes to have the centromeres of chromosome 14. Hybridization with D21Z1 showed that one of the der(14q21q) chromosomes also has the centromere of chromosome 21. Thus, one of the der(14q21q) chromosomes in the propositus was dicentric, while the other der(14q21q) was monocentric. The accessory chromosome 21 in the propositus had the normal centromeric hybridization pattern for chromosome 21 (Fig. 3C).

Based on these results, the karyotype of propositus was redesignated as 45,XY, der(14;21)(p11.1;p11.1)pat, der(14;21)(p11.1;q11.1)mat, +21mat [Mitelman, 1995].

PCR analysis on the index case using the polymorphic microsatellite marker TCRD, localized to 14q11.2, showed 2 distinct alleles, one inherited from the mother and the other from the father (Fig. 4). For chromosome 21, 10 markers were used for PCR analysis. However, D21S1259 was the only marker informative for the 3 chromosome 21s in the propositus. Of the 3 D21S1259 alleles seen in the propositus, 2 were inherited from the mother and one from the father (Fig. 5).

DISCUSSION

DS resulting from homozygosity for Robertsonian translocations der(14q21q) and an additional chromosome 21, though rare, has been reported [Borgaonkar, 1993]. Rockman-Greenberg et al. [1982] reported an abortus with homozygous (14q21q) translocation. Interestingly in that case, only the father carried the Robertsonian translocation, thus prompting the authors to speculate that a de novo (14;21) translocation arose in the germ cells of the mother. In that case, the parents elected to terminate the pregnancy and autopsy of the fetus showed no phenotypic abnormalities.

Homozygosity for a der(14q21q) can also result when both parents are balanced carriers of der(14q21q) and each parent selectively contributes the der(14q21q) at fertilization. It can also result if one parent carries such a translocation and if, subsequent to the splitting of the centromere during meiosis II, the 2 chromatids move to the same pole. Such a nondisjunction will result not only in homozygosity but also in uniparental disomy for the der(14q21q). Our investigations using cytogenetic heteromorphisms, FISH, and microsatellite polymorphism analyses indicated the biparental inheritance of the 2 der(14q21q) chromosomes in this patient.

Even though both parents were carriers of der(14q21q), in situ hybridization patterns of chromosome 14 and 21 alpha satellite probes indicated differences in the pericentromeric regions of the 2 der(14q21q) chromosomes. In the father, der(14q21q) was dicentric with the centromeres of both 14 and 21. The mother's der(14q21q) was monocentric and had the centromere of chromosome 14. In view of this information, we propose the following hypothesis to explain the selective nondisjunctional event in the mother. During maternal meiosis I, lack of a 21 centromere on the mother's der(14q21q) prevented meiotic pairing between the two 21s, assuming that pairing starts with the centromere. This lack of recognition of the chromosome 21 material on the der(14q21q) chromosome resulted in the anaphase movement of the free 21 along with the derivative chromosome resulting in 2 copies of 21. The father's der(14q21q), being dicentric, allowed the normal pairing with the free 21, followed by normal diplotene, diakinesis, and resolution of chiasmata. Subsequently, normal segregation occurred during anaphase 1, resulting in the der(14q21q) going to one pole and the free chromosomes 14 and 21 going to the other. With the increasing advances in the field of molecular genetics, it might be possible to test our hypothesis regarding failure of meiosis pairing using molecular markers. This proposed hypothesis is supported by the

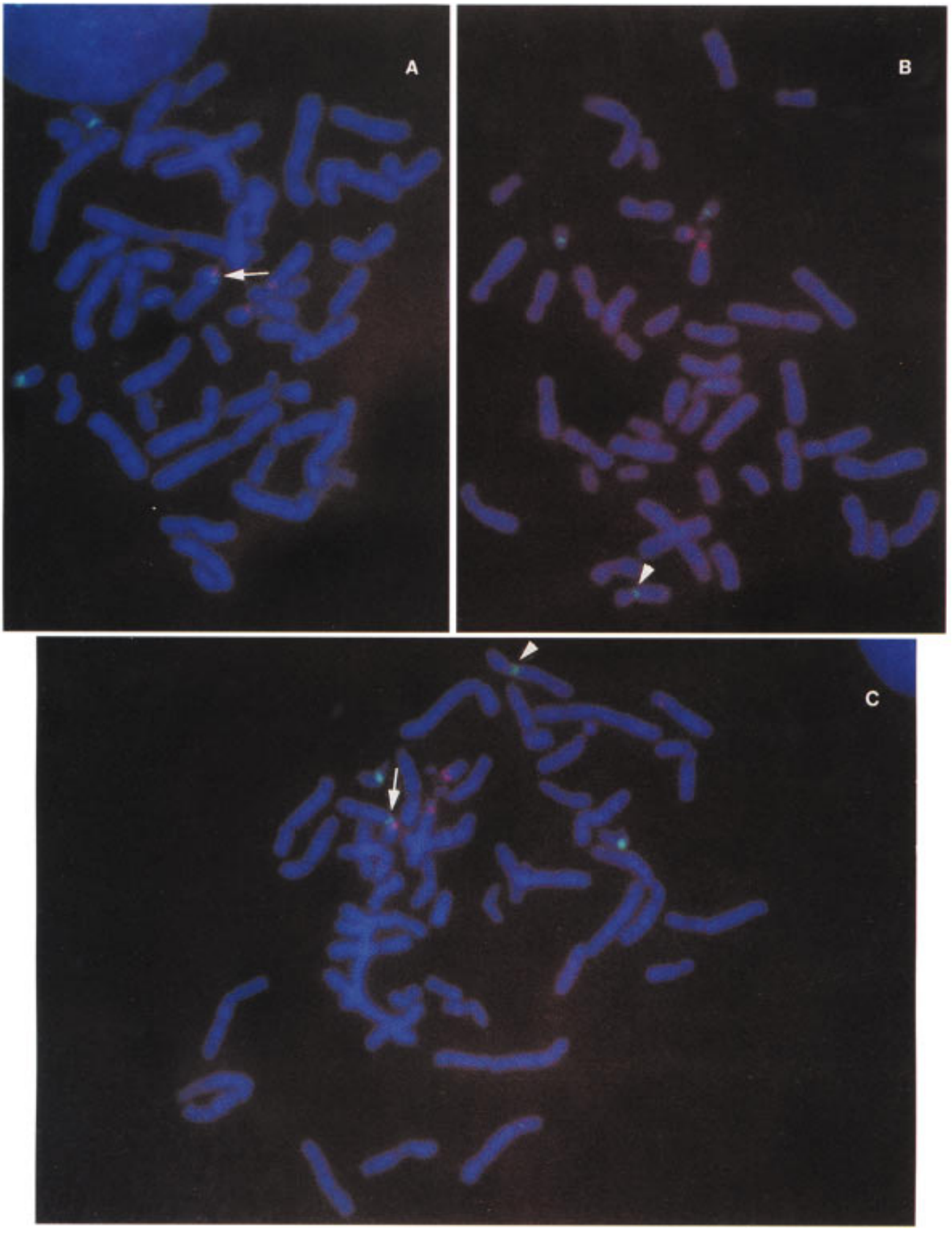

Fig. 3. FISH analysis using the alpha satellite probes for chromosomes 13/21 and 14/22. The probe for 13/21 was labeled with digoxigenin and detected with rhodamine; the probe for 14/22 was labeled with biotin and detected with fluorescein avadin. **A:** Father, the der(14q21q) is marked by an arrow. **B:** Mother, the der(14q21q) is marked by an arrowhead. **C:** Propositus, the paternally derived der(14q21q) is marked by an arrow and the maternally derived der(14q21q) is marked by an arrowhead.

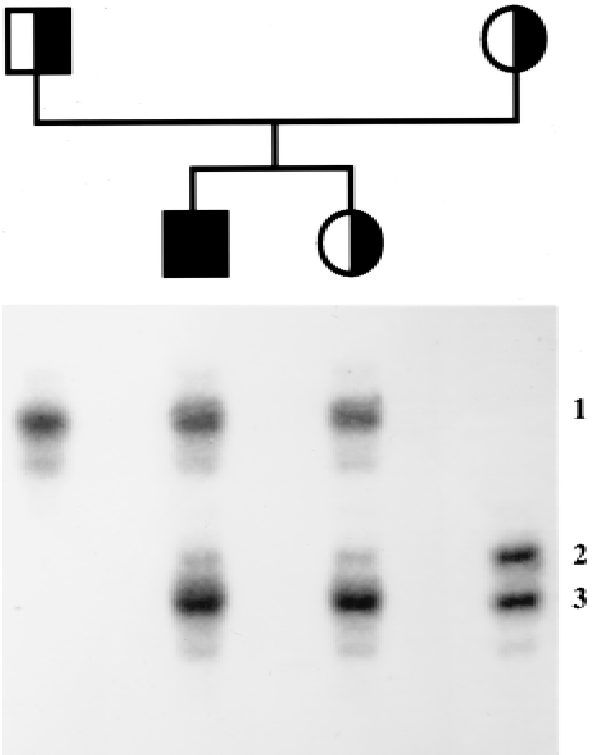

Fig. 4. PCR analysis using the microsatellite marker TCRD on chromosome 14q11.2.

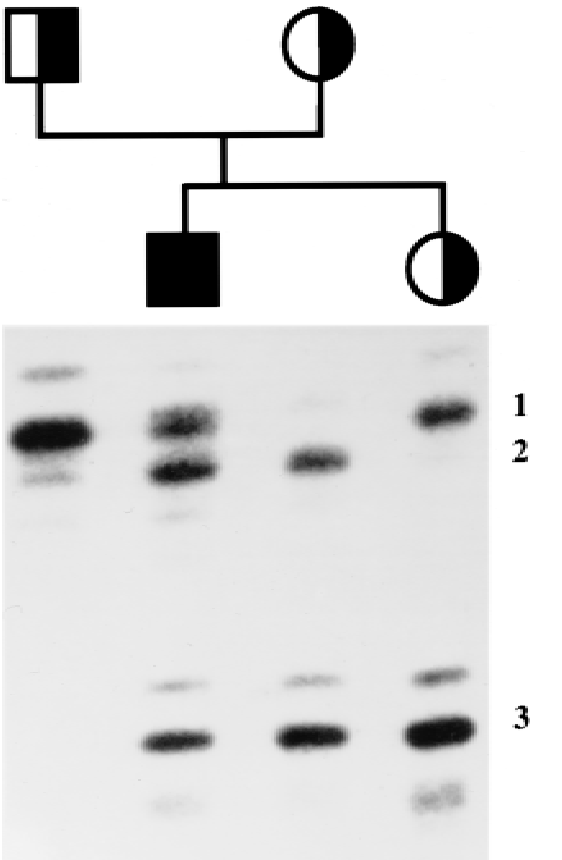

Fig. 5. PCR analysis using the microsatellite marker D21S1259 on chromosome 21q22.3.

observations that the nondisjunction risk may depend on the origin and structure of the centromeric region of the Robertsonian translocation [Stene and Stengel-Rutkowski, 1988]. Based on segregation analysis, these authors suggested that if the centromere in a t(14q21q) translocation originates from chromosome 14 or in a dicentric dic(14;21)(p11;p11), the 21 centromere is suppressed, leading to a segregation in which the Robertsonian translocation and chromosome 21 are included in a daughter cell. This corresponds to adjacent-1 segregation with separation of homologous centromeres. If, instead, the centromere originated from chromosome 21, or the 14 centromere was suppressed, there would be adjacent-2 segregation, resulting in nonseparation of homologous centromeres. Since, in our case, the centromere in the der(14q21q) in the mother originated from chromosome 14, adjacent-1 segregation resulted in the transmission of both the Robertsonian translocation and free chromosome 21 in the propositus.

From the combined results of classical cytogenetics, FISH, and microsatellite analyses, it is evident that the nondisjunction occurred during maternal meiosis I. The incidence of der(14q21q) in the general population is estimated to be low. The probability of such a translocation occurring in both the parents is even remote, except for consanguinity. In our case, the parents were nonconsanguineous. Microsatellite polymorphism analysis and FISH studies have shown conclusively that the parents were indeed unrelated and the 2 derivative chromosomes were not identical by descent.

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